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Human Papillomavirus Vaccination: Biology, Challenges, and Future Prospects

Elizabeth Duffy

ABSTRACT

Human Papillomaviruses (HPVs) are established as the most common sexually transmitted pathogens worldwide. Research has shown that vaccination against the virus pre-infection prevents most HPV-linked diseases. There are a variety of examples of these. Cervical cancer is the disease most commonly linked with HPVs, however they are also linked with a variety of other cancers. These include cancers of the male and female genitalia, as well as the head, neck, skin and oropharynx. Largely, these are caused by "high-risk "HPV types. Examples include HPV 16 and 18. Other types of HPV cause benign tumours (warts) in these regions. These are classed as "low-risk"and include HPV 6 and 11.he majority of these pathogenic strains are key ingredients in the three current vaccines. These are Gardasil-4, Cervarix, and Gardasil-9. However, a minority (30%) of HPV-linked diseases are caused by non-vaccine strains. This leaves even those vaccinated at risk of infection and hence disease. This highlights that more broadly protective vaccines are an urgent requirement for global eradication of HPV transmission and disease. This review aims to first explore key principles of HPV`s virology and their applications to current vaccinology. Challenges to these are then addressed followed by further exploration of these principles for prevention purposes.

Keywords: HPV, cervical cancer, background, factors, biology, human papillomavirus

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Conflict of Interest—I have written articles for the Irish Medical Times and was paid €900 for doing so. I also write on my own blog on vaccines and the issue of vaccine hesitancy which I hope to add advertisements, affiliate links and an online shop to in time. I also worked at AbbVie (a pharmaceutical company) for several months after graduating college| Funding—none

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INTRODUCTION

Human Papillomaviruses (HPV) are the most common sexually transmitted pathogens worldwide. Therefore, HPV transmission and infection causes a large-scale disease burden. There are a variety of common HPV-linked cancers.[1] 80). Across these areas benign warts are common on the genitals, oropharynx, hands, and feet. [1]Over 295,000 HPV-linked cancer deaths occur worldwide annually [2]. Various HPV-linked cancers have differing contributions to this burden. Cervical cancer is responsible for the largest proportion of these. More than 570,000 cases occur yearly, most (90%) are HPV-linked [2]. From these disease incidences, 311,000 deaths occur annually. [4] In contrast, oral cancers are responsible for approximately 145,328 deaths every year. [5] Head and neck cancers account for more than 563,826 cases per year including 274,850 oral cavity cancers, 159, 363 larynx cancers, and 52,100 oropharyngeal cancers.[6].

HPV case numbers and prevention strategies in Ireland

HPV-related disease is a significant burden in Ireland; and primary prevention strategies are in place to reduce it. Each year, 420 HPV-related cancers are diagnosed in Ireland every year; resulting in up to 130 deaths [7]. Over 6,500 women are hospitalized for precancerous growths in the cervix [8]. Primary strategies involve vaccination of girls in the first year of primary school, implemented since 2010, with gender-neutral vaccination from September 2019 [9]. The latter approach involves population based cervical screening of all women aged 25-60y [10]. In Ireland CervicalCheck is the cervical screening programme, which offers cervical screening for all women aged 25-60 [11]. In March 2020, cervical screening in Ireland switched from a cytology based test to a molecular HPV test [12]. Early detection of cervical abnormalities is vital to enhance the potential for survival for these patients.Over 100 HPV subtypes have been identified to date, 40 of which infect the genital tract [13]. 14 of these have been identified as carcinogens; and subdivided into high and low-risk types [14]. High-risk types include HPVs 16,18, 31, 35, and 51 [15] and were responsible for over 600,000 cancer cases globally in 2008 alone. [16]. These types commonly cause dysplasias and squamous cell cancer in the anogenital and oropharyngeal regions [17,18], Notably, HPV 16 and 18 are linked for the majority of invasive cervical cases (70%)worldwide.[19]. Low-risk types include HPVs 2, 6, and 11 [20,21] . The first is a common causative agent of benign skin warts (verruca vulgaris) [22]; the last two of genital condylomas, and anogenital warts [22]Non-vaccine strains cause a small percentage (30%) of HPV-linked disease worldwide, and rarer strains within this subclass are often underdiagnosed [23, 24].These include HPV 30, 91, and 74, which have been shown to contribute to cervical lesion development [24]. The knowledge that certain HPV strains are oncogenic has transformed strategies used to control and manage HPV-linked cancers. The two major preventative strategies used worldwide are screening and vaccination.[25] The three currently licensed vaccines in use are the bivalent Cervarix, Gardasil-4, and Gardasil-9 [26].The goal of this review is to discuss key principles of HPV biology and how they apply to the development and use of current vaccines. It will also discuss how these principles might be further developed to overcome current challenges. These include inadequate supplies of vaccines in low to middle income countries (LMICs), and maintaining a high uptake rate of vaccines.

Principles of HPV Virology

3.1 Structure

Human Papillomaviruses are a large family of small, icosahedral, non-enveloped viruses containing a histone-associated circular dsDNA genome [27-29] They have a diameter of 50-55nm [30, 31]. This compact nature is due to the size and orientation of its major and minor structural proteins: L1 and L2 [32, 33]. It was discovered in 1993 that spontaneous self-assembly of the mature capsid structure occurs when L1 is expressed alone, or co-expressed with L2 [30]. This property is key for design and efficacy of vaccines against a variety of HPV types [34-36], which will be discussed in more detail . This mature product of self-assembly is depicted in Figure 1.

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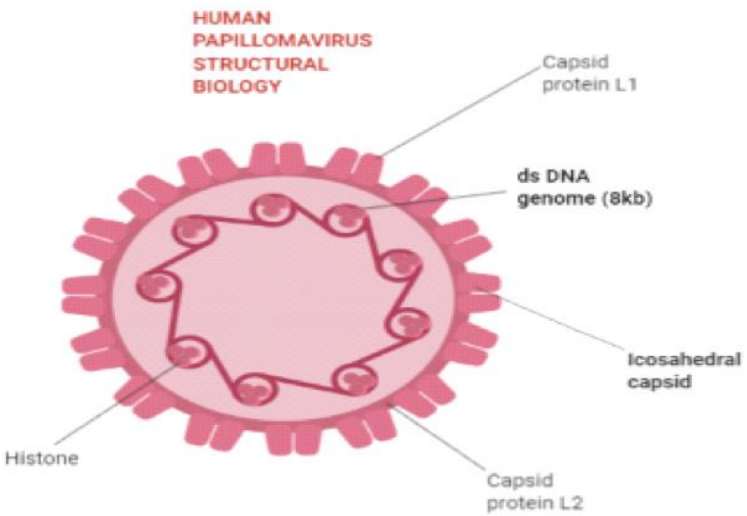

Fig 1: Schematic of HPV`s structural biology: HPVs are a large family of icosahedral, non-enveloped viruses [27-29] with a virion diameter of 50-55nm [30, 31] Its capsid is composed of the major and minor structural proteins; L1 and L2, respectively [32, 33]. These two proteins self-assemble into a compact, icosahedral structure, encapsulating a histone-associated circular, dsDNA genome [27, 28, 30, 37].

Genetics

Control over the sequence of protein transcription is important for a successful infection. The organization of the viral genome is key to regulating protein expression. HPV genomes, depicted in Figure 2, are histone-associated, double-stranded, circular DNA genomes measuring 8kb [27, 37, 38]This genomic content is subdivided into 8-9 protein-coding open reading frames, with the distribution of protein-coding sequences defined by exon-intron boundaries. [39, 40] This arrangement defines three functional regions , an upstream regulatory region, the early region, a late region and a noncoding region/LCR [40].A key feature of the Upstream Regulatory Region (URR)/Long Control Region (LCR) is being flanked by L6 and E1. It also contains the origin of replication and recognition sequences for cellular and viral transcription factors [41, 42] The early region encodes six genes, four of which are involved in replication, transcription, and manipulation of the cellular milieu [40, 43] The highly conserved E1 and E2 proteins regulate early viral transcription, by acting as sequence-specific origin recognition factors [44-47].

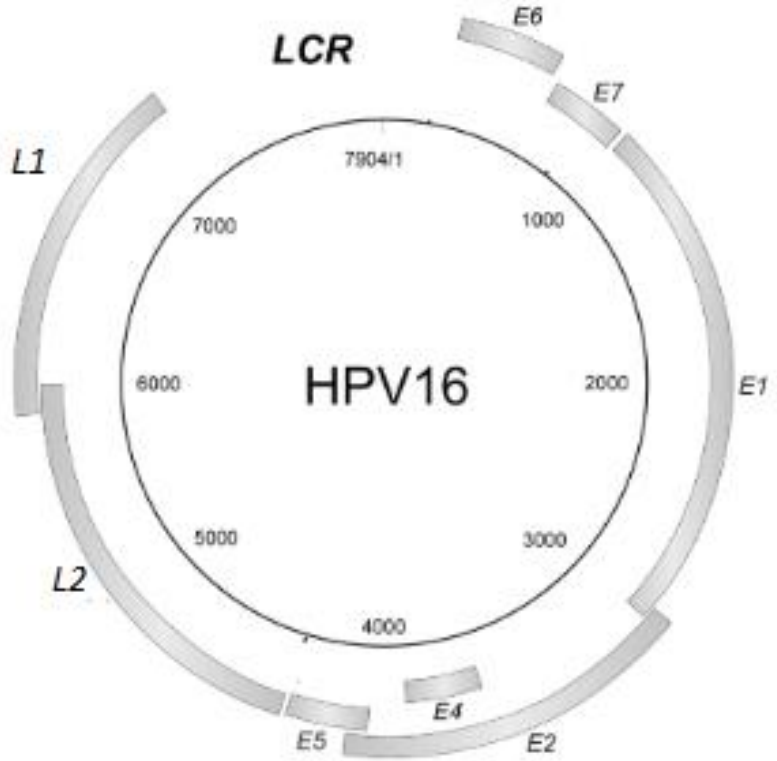

Fig 2: Diagram of the genome of HPV 16: HPV genomes are circular dsDNA genomes with genomic content reaching 8kb[27, 37, 38]. This is subdivided into three key functional regions; the Upstream Regulatory Region (URR)/Long Control Region(LCR), as well as the early and late regions [40, 48]. The URR/LCR is flanked by L1 and E6 [41], adjacent to its partner oncoprotein E7. Further downstream from this are the E1-E5 proteins. The late region encodes the structural L1 and L2 proteins [40] . Image adapted from Kajitani et al, 2012. All copyright to Kajitani et al, I do not own this image.

Life cycle

The HPV life cycle is tightly linked with the differentiation status of basal columnar epithelial cells. [49-51] These form a layer of stratified squamous epithelial tissue, located directly above the basement membrane. [51-53] They are not directly accessible to virions circulating in the cervical microenvironment. Thus, the main access route to target cells is micro-traumas to the cervical epithelium, including micro-wounds, cuts, or other micro -abrasions. [52, 54]

Infectious mechanism

High-risk HPV infection of basal keratinocytes, squamous and stem cells, as well as other epithelial cell types can be divided into three phases

1.Cellular attachment and endocytosis

Host cell attachment and endocytic mechanisms vary among HPV genotypes and other DNA viruses [55 56 57] . Tumorigenic and phylogenetically related strains bind ECM-associated syndecan-1 and laminins [58 ,59]. Several in vitro studies have also demonstrated that laminin-5 can mediate binding to the ECM in several HPV types [60 , 61]. Cyclophilin-B catalyzed conformational changes expose the highly conserved L2 N-terminus for furin-catalyzed cleavage [62]. The mature cleavage product allows infectious endocytosis [62]. Data from 2012 in HeLa and HaCaT cells reveals that HPV16 uses a ligand-induced endocytic pathway related to macropinocytosis [63]. Data varies among cell lines and genotypes, but oncogenic strains share cellular factors in their endocytic pathways. Bovine papillomavirus uptake occurs via a clathrin-dependent pathway [64].

1. Genome amplification

Early intracellular phases were investigated in 2002 in low-passage number human foreskin keratinocytes, and immortalized adult skin cell lines [65]. Using these, reverse transcription and RT-PCR detected spliced E1 and E2 mRNA transcripts 4 hours post infection; and the other viral proteins up to 10 hours post infection [65]. The translated E1 and E2 viral proteins then interact in vivo,resulting in a multimeric E1ori initiation complex [66]. They are the major virally-encoded factors allowing transitory replication of ori-containing viral episomes [67] . The 8kb episomes have limited coding capacity, and their replication depends on host-encoded factors [38] , [68]. These are recruited to replication foci in an E1-dependent manner, synthesizing 50-100 episomal copies per nucleus [50].

Virion release

L2 expression, and E2-medicated recruitment to replication sites concludes the intracellular portion of the HPV life cycle [52]. L1 proteins self-assemble into pentameric capsomeres and are transported to the nucleus [69] Newly synthesized viral genomes are located in the PML, adjacent to viral capsid proteins [70]. Once all components are recruited, capsid assembly occurs [71]. Mature virions are shed from the most superficial epithelial layer inside dead squames, and infect neighboring cells [50].

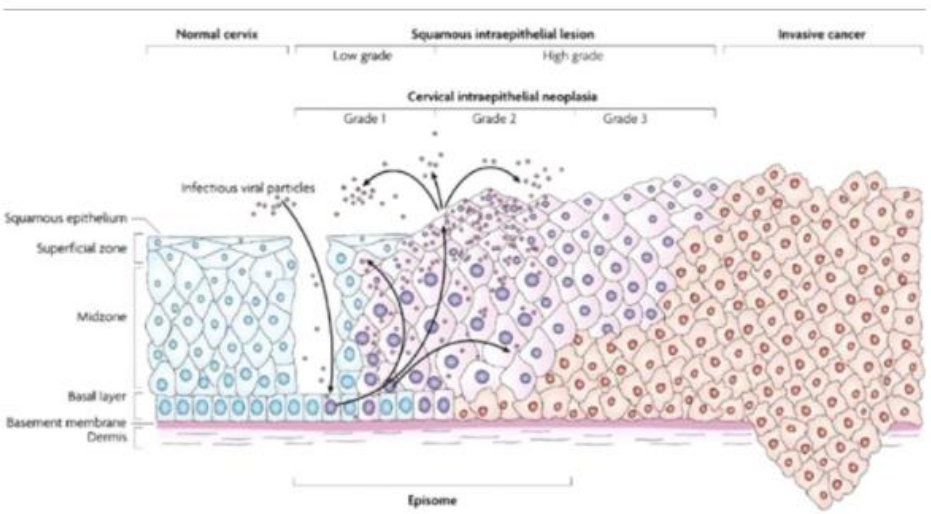

Fig 3: Schematic of HPV infection progression: HPV infects columnar cells forming the basal layer of the cervical squamous epithelium. [1], [72] It accesses these through micro abrasions or wounds [40] arising from sexual intercourse[73]. Initial establishment of infection is followed by a maintenance phase of viral replication [1],[74], at which viral DNA is maintained at a low but constant copy number[40, 2, 74]. On reaching the epithelial surface, viral DNA is replicated to a high copy number, and the late proteins L1 and L2 are expressed to a high level[75]. Progeny viral particles are finally released from the epithelial surface[75]. These continue the infectious cycle in neighboring cells.

Natural History of HPV Infection

Viral clearance occurs in (90%) of newly acquired infections within 1-2 years [76]. However, this apparent clearance of the virus may result from the viral load being reduced to sub detectable levels by the immune system[77]. This maintains the virus in a state of latency, elegantly described by Paul Lieberman as "a metastable, nonproductive infection state that is capable of subsequent reactivation to repeat the infection cycle"[78]. Underlying immunological mechanisms vary with antigenic types and the interval of time between infection and immune detection [79]. However, key features are low-level viral genome expression in basal epithelial cells, and a resultant lack of antigen detection by immune cells [79]. After presumed viral clearance, such latent infection persists ina small percentage (10-20%) of women [80] , [81] , [82] Cervical cancer develops over a timeframe of up to 2 decades [83]. It follows that long-term HPV infection can lead to precancerous

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neoplasias, CIN grade 1,2, and 3, resulting in invasive cervical cancer [84].

Disease stage is graded based on patterns of microscopic organization of cells in tissue samples from cervical biopsies or other surgical specimens [85]. Among specimen types, the key diagnostic feature is the proportion of the cervical epithelium occupied by undifferentiated basal cells. The risk of malignancy development increases with the number of undifferentiated cells found at diagnosis. CIN1 manifests histologically as dysplastic columnar cells restricted to the lower third of the cervical epithelium [86]. Higher-grade epithelial abnormalities result from CIN1 in a small percentage (1.5-10%) of cases; with some studies finding no malignant progression [87]. CIN2 progression biology is less well understood [88]. However, it is characterized by dysplastic changes localized to the lower 2/3 of the cervical epithelium, marked nuclear abnormalities, and stretched stromal capillaries [86]. This increased cytopathy causes higher progression rates compared to CIN1. These vary (10-40%) among studies with factors including age, country, and pregnancy [88-90]. CIN3 is a direct precursor to cervical cancer; with approximately one-third (33%) of advanced lesions resulting in a cervical cancer diagnosis within 20 years from time of infection [91].

Based on these principles, three major vaccines have been developed and approved to date.

Gardasil-4

According to the official website of the European Union, Gardasil was authorized for marketing in the EU on the 20th September 2006 [92]. It is licensed to prevent HPV-linked diseases in both men and women from the age of nine through 26 [92, 93]. These include precancerous growths in the cervix, vulva, vagina, anus, and cervical and anal cancers. [92]

This multi-tissue effectiveness can be understood by outlining key vaccine ingredients, as well as immunological properties and their effects. Across the literature, the formulation is described as containing "VLPs of the recombinant major (L1) capsid protein of HPV types 6,11,16, and 18", self-assembled into arrays of 72 pentamers [93, 34]. This highly repetitive surface structure facilitates interaction with innate humoral immune system components, allowing APC – mediated opsonization and phagocytosis [94]. Addition of 225mcg of aluminium promotes antigen uptake by dendritic cells at the site of injection, and their subsequent maturation [93, 95].

Cervarix

Enhanced immunogenicity was a feature of Cervarix, licensed in 2007 [96]. It is authorized in the European Union for males and females from the age of 9 to 25 to prevent cancers of the cervix and anus, as well as precancerous lesions in the cervix, vulva, vagina, or anus [97].

This also has synthetic VLPs containing L1 epitope of HPV 16 and 18 [98]. However, extra immunogenicity derives from the addition of a novel adjuvant called AS04 (Adjuvant System 04) [99]. It is based on a TLR4 agonist, MPL (3-O-desacyl-4'-monophosphoryl lipid A), combined with aluminium salt [100]. The combination product binds and activates Toll-Like receptors in the same way as a biological agonist or PRR would [99]. The result is an intracellular cascade yielding enhanced immunogenicity at both the cellular and humoral level. T cells are activated, and antibodies are retained in the blood long-term [99].

Gardasil-9

Gardasil 9 was authorized for marketing in the EU on the 9th June 2015. [101] Like Gardasil, it is licensed to prevent a variety of HPV-linked maladies in both men and women from the age of nine. [101] The include precancerous lesions and cancers in the cervix, vulva, vagina, anus, and genital warts. [101]

Broader protection was established with the release of Gardasil-9 in 2014 [96]. The basis of this is the inclusion of L1 epitopes from a wider variety of strains. As well as including the most common cervical oncogenes HPV 16 and 18 [102] . Gardasil-9 has high-risk strains associated with other HPV-linked cancer types. These include HPV 31, 33, 35, 45, 52 and 58 [102]. Two benign strains are included, HPV 6 and 11 [102]. Cervarix has the smallest antigenic concentration of all the vaccines, and contains the AS04 adjuvant for increased immunogenicity [103 98].

In summary, Gardasil-9 provides prolonged immunity to a wide range of HPV-linked diseases.

Gardasil-9 also differs from the other vaccines in ingredient concentration as well as composition. Specifically, it contains twice the concentration of aluminium as well as L1 protein from HPV 16 and 18 [98, 103]. A comparison of ingredients in the various vaccines is provided in Table 1

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TABLE 1: Summary of HPV vaccine ingredients

Name of the vaccine	HPV Strains contained in it	Other ingredients
Gadasil-4	HPV 6,11,16 and 1B L1 proteins	Amorphous aluminium hydroxyl phosphosulfate adjuvant
Cervarix	HPV 16 and 18 L1 proteins	3-o-desacyl-4-monophosphoryl lipid A (MPL) Aluminium hydroxide Sodium chloride Sodium phosphate
Gardasil-9	HPV 8,11,16,31,33,45,52, and 58 L1 protein	Amorphous aluminium hydroxyl phosphosulfate adjuvant

Table 1: A summary of the ingredients of the three currently available HPV vaccines. These are Gardasil-4, Gardasil-9, and Cervarix. The first, released in 2006. is a quadrivalent vaccine containing recombinant L1 protein of HPVs 6, 11, 16, and 18 [92]. Another key ingredient is an amorphous aluminium hydroxy phosphosulfate adjuvant, which enhances the immune response [92, 104]. Cervarix, released in 2007, contains recombinant L1 proteins from HPV 16 and 18 [97]. The most recently released vaccine, Gardasil-9, contains L1 protein from HPVs 6, 11,16,18,31,33,45,52, and 58 [101]. It also contains the amorphous aluminium hydroxy phosphosulfate adjuvant [101].

Efficacy of HPV vaccines

In addition to these, larger randomized control trials have been carried out investigating population-level efficacy of HPV vaccination. A systematic review of 65 articles investigating the effectiveness of HPV vaccination was carried out.[105] It investigated countries using multi-and single cohort vaccination programmes.[105] Countries using a multi-cohort approach had a reduced prevalence of several high – risk HPV types and anogenital wart diagnoses 8 years after the introduction of HPV vaccination.[105] Further, a non-systematic review was carried out to investigate the burden of HPV-linked disease in Australia before and after introduction of HPV vaccination.[106] It reported significant declines in high-grade cervical disease among women after the introduction of HPV vaccination. [106] In addition, a reduction in genital warts and HPV infections was observed among men after introduction of male HPV vaccination. [106].

The impact of long-term vaccination strategies

There is good evidence for long-term vaccination strategies. After implementation of the National HPV Vaccination Programme in Australia in 2007, incidence rates of 4v HPV type infections declined significantly (28.7% to 2.3%) in the period from 2005 to 2012 [106]. Further, a 2021 study published by Partha Basu and colleagues investigated the efficacy of one, two and three doses of quadrivalent HPV vaccination against persistent HPV 16 and 18 infection, the HPV genotypes responsible for the majority (70%) of cervical cancer cases [107]. The study was carried out in Indian girls aged 10-18, over 4,000 of whom received a single dose [107]. The study demonstrated very high efficacy of just one dose of the quadrivalent vaccine against persistent infection by HPVs 16 and 18 [107]. Notably, such protection was maintained until 10 years following vaccination [107]. No difference in efficacy was noted between one, two, and three doses of the quadrivalent vaccine [107]. Recent studies have found similar results among multiple cohorts a decade after single-dose quadrivalent vaccination [108, 109].

Challenges associated with current HPV vaccines

Therefore, evidence supports vaccination being an effective prevention strategy against both high and low-grade HPV-linked disease among many cohorts. However, this efficacy may not extend to the many non-vaccine strains.

Only 9 of the known 200 HPV strains are included in current vaccines; with some degree of cross-

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protection [110].Of these, only two are included in Gardasil 4 and 9 [92, 101]. These are the high-risk strains HPV 16 and 18; which together are responsible for the majority (70%) of cervical cancer cases . Gardasil-4 can also protect against low- risk strains [92, 111]. The specific strains are HPV 6 and 11, which together are responsible for most cases (90%) of anogenital warts [112]. According to the CDC, these strains are commonly identified before or on detection of anogenital warts[112] . Gardasil-9 has 5 additional strains [101]. This expands its strain coverage to include HPVs 31,33, 45, 52, and 58 [101]. However, it is unlikely that there will be a linear decrease in cervical cancer incidences with increased vaccination. Many non-vaccine strains have been linked with disease, known as "low-risk HPVs" which may contribute to the disease burden. There are over 200 identified types of such prevalent lrHPVs , the majority of which are not included in the ingredients of the currently available vaccines [111 92, 97, 101]. Infection with such strains is associated with a minor (1-3%) risk of cancer development if not resolved [111].

HPV-linked disease in developing countries

HPV-linked disease is a significant burden in developing countries compared to more developed countries. Among these, the epidemiologic distribution of HPV morbidity and mortality can vary significantly. According to a 2018 study published by Aamod Dhoj Shrestha and colleagues, cervical cancer rates are " highest in Eastern Africa (including Zimbabwe) and lowest in Western Asia [113]. It is the second most common type of cancer in women in the South East Asia region and a major cause of cancer deaths among women of low and middle income countries (LMICs) like Nepal." [113] In low HDI countries such as Ethiopia, a majority (90.2%) of cervical cancer cases are attributed to HPVs 16 and higher (18-20%) than the global average [114]. Despite this, modelling analyses have predicted that high HPV vaccination coverage in LMICs can result in cervical cancer elimination in the majority of LMICs by the end of the century [115].

However, why hasn`t this happened? There are cultural and socioeconomic factors causing this disparity in disease incidences. There is limited awareness about HPV, and HPV-linked disease. A key factor contributing to this is that discussion around reproductive health can be stigmatized in developing countries [116]. HPV vaccination in particular, is stigmatized. There is a notion that it will lead to adolescents being irresponsible around their sexual health, which is not supported by studies [116] Although the cost of the vaccine product itself is generally equivalent to that of other childhood vaccines; delivery costs generally exceed that of other vaccines [117]. In addition, introduction of other vaccines may be prioritized, including rotavirus and pneumococcal vaccines. [118] A further factor is limited access to secondary prevention strategies such as screening in LMICs[119]. Therefore, a key factor limiting vaccination coverage is a lack of vaccine supply to LMICs. According to the Global Market study of HPV vaccinations published by the WHO in 2019, the global demand for HPV vaccines surpassed production capacity in 2017, as well as ongoing efforts to scale up vaccination production.[120]

Vaccine Hesitancy

Vaccine hesitancy has become an increasingly important contributor to recent drops in global vaccination rates seen. In 2018, the CDC reported an increase in the number of children unvaccinated at 2 years old. Further, in March 2020, it was found that over a third of US citizens between 19 and 35 were not vaccinated[121], and a 2019 national survey reported that 1 in 4 parents had serious concerns about vaccinating their children [122]. Thus, addressing concerns raised in the media about vaccines will be essential to restore public confidence in vaccines and thus increase uptake rates [123].

Conclusions

In summary, structural, genetic, and life cycle aspects of HPV`s biology have been well established. This knowledge has been harnessed to develop current vaccines against it. These have proven highly efficacious across a variety of clinical trials. This derives from their key ingredients: Virus-Like Particles, which are the ideal combination of immunogenic and nontoxic. These are made from the spontaneous self-assembly of recombinant L1 proteins, which are overexpressed in a host cell.

L1 proteins from only a small subset of HPV types are included. However, these vaccines also cost hundreds of millions of dollars to develop, as well as

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being costly to transport. For this reason, broad protection and cost efficiency are essential features of any future HPV vaccines. Future vaccines must further optimize immunogenicity, safety, and cost-efficacy. This will decrease global transmission and hence disease rates.

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